

## New England Biolabs Certificate of Analysis

*Product Name:* T7 RNA Polymerase  
*Catalog #:* M0251S/L  
*Concentration:* 50,000 units/ml  
*Unit Definition:* One unit is defined as the amount of enzyme that will incorporate 1 nmol ATP into acid-insoluble material in a total reaction volume of 50 µl in 1 hour at 37°C in 1X RNA Polymerase Reaction Buffer.  
*Lot #:* 0201709  
*Assay Date:* 09/2017  
*Expiration Date:* 9/2019  
*Storage Temp:* -20°C  
*Storage Conditions:* 100 mM NaCl, 50 mM Tris-HCl (pH 7.9), 1 mM EDTA, 20 mM BME, 0.1 % Triton X-100, 50 % Glycerol  
*Specification Version:* PS-M0251S/L v3.0  
*Effective Date:* 25 Aug 2016

Assay Name/Specification (minimum release criteria)	Lot #0201709
<b>Endonuclease Activity (Nicking)</b> - A 50 µl reaction in RNAPol Reaction Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 150 units of T7 RNA Polymerase incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	<b>Pass</b>
<b>Exonuclease Activity (Radioactivity Release)</b> - A 50 µl reaction in RNAPol Reaction Buffer containing 1 µg of a mixture of single and double-stranded [ <sup>3</sup> H] <i>E. coli</i> DNA and a minimum of 150 units of T7 RNA Polymerase incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	<b>Pass</b>
<b>Non-Specific DNase Activity (16 Hour)</b> - A 50 µl reaction in RNAPol Reaction Buffer containing 1 µg of Lambda DNA and a minimum of 250 units of T7 RNA Polymerase incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	<b>Pass</b>
<b>Promoter Specificity</b> - A 50 µl reaction in RNAPol Reaction Buffer in the presence of 2 mM NTPs containing 1 µg of Lambda DNA as a template and a minimum of 200 units of T7 RNA Polymerase incubated for 1 hour at 37°C results in <1.5% of the amount of product incorporated as compared to a control reaction using T7 DNA as a template.	<b>Pass</b>
<b>Protein Purity Assay (SDS-PAGE)</b> - T7 RNA Polymerase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	<b>Pass</b>



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Assay Name/Specification (minimum release criteria)	Lot #0201709
<b>RNase Activity (Extended Digestion)</b> - A 10 µl reaction in RNAPol Reaction Buffer containing 40 ng of a 300 base single-stranded RNA and a minimum of 50 units of T7 RNA Polymerase is incubated at 37°C. After incubation for 4 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.	<b>Pass</b>

*M.W. Southworth*

Authorized by  
Maurice Southworth  
25 Aug 2016

*Dongxian Yue*

Inspected by  
Dongxian Yue  
21 Sep 2017

